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PORTABLE MULTIPLEX PATHOGEN DETECTOR

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ABSTRACT

Tumor marker concentrations in serum provide useful information regarding clinical stage and prognosis of cancer[1] and can thus be used for presymtomatic diagnostic purposes. Currently, detection and identification of soluble analytes in biological fluids is conducted by methods including bioassays, ELISA, PCR, DNA chip or strip tests. While these technologies are generally sensitive and specific, they are time consuming, labor intensive and cannot be multiplexed. Our goal is to develop a simple, point-of-care, portable, liquid array-based immunoassay device capable of simultaneous detection of a variety of cancer markers.

Here we describe the development of assays for the detection of Serum Prostate Specific Antigen, and Ovalbumin from a single sample. The multiplexed immunoassays utilize polystyrene microbeads. The beads are imbedded with precise ratios of red and orange fluorescent dyes yielding an array of 100 beads, each with a unique spectral address (Figure 1). Each bead can be coated with capture antibodies specific for a given antigen. After antigen capture, secondary antibodies sandwich the bound antigen and are indirectly labeled by the fluorescent reporter phycoerythrin (PE). Each optically encoded and fluorescently- labeled microbead is then individually interrogated. A red laser excites the dye molecules imbedded inside the bead and classifies the bead to its unique bead set, and a green laser quantifies the assay at the bead surface. This technology has been proven to be comparable to the ELISA in terms of sensitivity and specificity.[2]

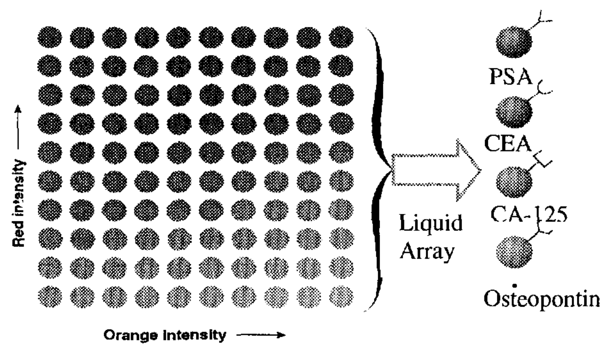

Figure: 1 Different antibodies on each bead enables deeply multiplexed detection

We also describe the laser-based instrumentation used to acquire fluorescent bead images Following the assay, droplets of bead suspension containing a mixture of bead classes were deposited onto filters held in place by a disposable plexiglass device and the resultant arrays viewed under the fluorescent imaging setup. Using the appropriate filter sets to extract the necessary red, orange and green fluorescence from the bead array, digital images were captured to computer with a Pixel CCD camera which were subsequently analyzed using customized digital image processing software.

MATERIALS AND METHODS

REAGENT PREPARATION:

Different sets of carboxylated fluorescent microspheres were obtained from Luminex Corp, Austin, TX. Capture antibodies were covalently coupled to a unique carboxylated bead set (1.25 X 10^6 microspheres in 100 µL) in accordance with the manufacturer's protocol. The end product is a mixture of several bead classes coated with different target proteins. Rabbit Anti-Ovalbumin, Rabbit Anti-Bacillus Globigii (Bg), Rabbit Anti-MS2 were purchased from Tetracore (Gaithersburg, MD). Monoclonal Antibody to PSA and Rabbit Anti-human PSA were purchased from Biodesign Int. (Saco, Maine). The detector antibody cocktail comprised a mixture of the 3 biotinylated antibodies viz. RaOv, RaMs2, RaBg for the respiratory panel and mAPSA, RaPSA and RaOv for the cancer panel at a final concentration of 3 µg/ml. Antigen solutions were prepared in PBS, pH 7.4. Bg was obtained from Dugway Providing Ground. MS2 was obtained from ATCC, Ovalbumin was purchased from Sigma and Prostate Specific Antigen (ACT complex and 98% pure) from Biodesign International.

MICROSPHERE ASSAY PROTOCOL:

Bead solution was incubated with 100 µL sample for 30 minutes at ambient temperature. The mixture was vacuum aspirated, washed 2X with 100 µl buffer to remove unbound antigen and resuspended in 100 µL PBS-TBN. 50 µL of the biotinylated antibody solution was added to the bead mixture, and incubated 30 minutes. The mixture was vacuum aspirated, washed to remove excess detector antibody and resuspended in 100 µL PBS-TBN. 50 µL SA-PE was added and the reaction mixture incubated 5 minutes. The mixture was vacuum aspirated, washed, and resuspended in 100 µL PBS-TBN. The beads were then transferred to a plexiglass disposable chamber onto a thin flat filter ready for imaging.

INSTRUMENTATION.

Using appropriate laser filter sets to extract the necessary "red-orange" image pair for classification and a "green" image for reporting the level of attached antibodies, digital images of the bead array are captured following laser excitation using a Pixel CCD camera and processed on a PC.

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